Evidence of a dual histogenetic pathway of sacrococcygeal teratomas

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Abstract

Aims: Sacrococcygeal teratomas are rare tumors that occur most frequently in neonates, although adult cases also occur. Molecular pathogenesis of these tumors and long term outcome is uncertain.

Methods and results: Fifty four sacrococcygeal teratoma specimens from 52 patients were identified and available follow up information was obtained. Fluorescent in situ hybridization analysis was performed to identify i(12p) abnormalities on paraffin blocks of the tumor. Among the 48 pediatric patients, there were 44 teratomas and 4 tumors with teratoma and yolk sac tumor (1 of which also had primitive neuroectodermal tumor). The teratomas included 37 mature teratomas and 11 immature teratomas (4 grade 1, 2 grade 2, and 5 grade 3). The 44 teratomas lacking a yolk sac tumor component were all negative for i(12p). The 4 tumors with a yolk sac tumor component were all positive for i(12p). The 4 adult cases all lacked nonteratomatous germ cell tumor components, immature elements, and i(12p). Follow up information was available for 32 patients. Two patients with teratoma had recurrence, but were alive with no evidence of disease after long-term follow up. One patient with teratoma and yolk sac tumor had recurrence 7 months after resection. The other patients were alive with no evidence of disease at last follow up.

Conclusions: Our data suggest that pediatric sacrococcygeal teratomas should be considered as two distinct groups with a divergent histogenetic pathway. Prognosis of these tumors are excellent, despite rare recurrence.

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Introduction

Extragonadal germ cell tumors (GCTs) are rare tumors and accounting for 1-6% of all GCT cases.[1-5] Extragonadal GCTs are thought to arise from germ cell precursors that remain along paths of embryonic migration and form tumors in the sacrococcygeal region, retroperitoneum, mediastinum, or other sites. [4] Although the morphologic appearances are analogous to those in the gonads, the frequency of GCT types and patient distribution are different. The most common extragonadal GCT location in adults is the mediastinum, whereas the sacrococcygeal region and central nervous system are more common sites in children.[6-10] The behavior is dependent on the combination of patient age, histology type, anatomic site, and clinical stage.[4]

Pediatric sacrococcygeal teratomas most often occur in neonates. Benign behavior is observed in most cases, although metastatic disease has been reported in a fraction of cases. The presence of immature elements in neonatal teratoma is not associated with malignant behavior, but the presence of a nonteratomatous component (yolk sac tumor and/or embryonal carcinoma) is considered evidence of malignant potential and an indication for three-agent cisplatin-based chemotherapy,[11, 12] although patients with α-fetoprotein and β-human chorionic gonadotropin measurements that return to normal following surgery have been followed without chemotherapy ("watch and wait" approach) by some groups.[13-15] In the absence of nonteratomatous elements, local recurrence may occur, but up to 100% long term survival has been described in sacrococcygeal teratomas, even in the presence of immature teratoma.[16] Very rare sacrococcygeal teratomas may recur as YST, but this may represent inadequate histologic sampling of the primary tumor.[17, 18] Sacrococcygeal tumors with a yolk sac tumor component have, even when metastatic, a relatively good prognosis with chemotherapy.[13, 15]

Adult sacrococcygeal teratomas are very rare, with fewer data regarding prognosis, but the surgical treatment of adult sacrococcygeal teratoma has recently been discussed.[19] Metastasis was observed in 3 patients in a series of 26 cases (12%), exclusively in women.[19] Associated somatic-type malignancies are associated with poor prognosis.[19, 20] Somatic-type carcinomas include adenocarcinoma, adenosquamous carcinoma, and low-grade neuroendocrine carcinoma.[19]

The isochromosome 12p abnormality is typical of postpubertal testicular germ cell tumors, but is lacking in germ cell lesions with expected benign behavior, such as pediatric testicular teratoma and ovarian mature cystic teratoma. Malignant GCTs in postpubertal patients, regardless of location, often have isochromosome i(12p).[21-26] However, the same genetic abnormality is extremely rare in pediatric cases, even those with yolk sac tumor.[21, 27, 28] Instead, the genetic alterations in malignant GCTs of prepubertal patients are frequently gains of chromosomes 1q, 3, or 20q and loss of chromosomes 1p, 4q, or 6q.[29-31]

Sacrococcygeal teratoma is the most common GCT of neonates and rarely occurs in adults, [16, 20, 32, 33] yet studies of their i(12p) status are extremely limited except for one recent study.[16] We investigated the i(12p) status of a large number of primary sacrococcygeal teratomas in both children and adults, including cases with malignant germ cell tumor elements (primitive neuroectodermal tumor and yolk sac tumor).

Materials and Methods

A computer-based text search of the surgical pathology files of Indiana University was conducted for “sacrococcygeal” and “teratoma” from 1990 to 2014, and we identified all specimens with paraffin blocks permitting the investigation of chromosomal 12p abnormalities

by fluorescence in situ hybridization (FISH). These included 4 specimens from adult patients and 50 specimens from 48 pediatric patients. Clinical information including patient sex, age, duration of available follow up, and patient status at last follow up was obtained. All hematoxylin and eosin-stained sections of the tumors were reviewed by two pathologists (CK and LC) to confirm the diagnosis of teratoma and evaluate the following: histologic grade of teratoma using the same grading system as that used for ovarian teratomas and presence of malignant GCT.[34, 35]

FISH testing was performed as previously described.[36] The slides were deparaffinized with two 15-minute washes of xylene, followed by two 10-minute washes of absolute alcohol. The slides were then air-dried in a fume hood. The slides were treated in 0.1 mM citric acid (pH 6.0; Zymed, Carlsbad, CA) at 95°C for 10 min, rinsed in distilled water for 3 min, and washed with 2 x SSC for 5 min. Digestion of the tissue was performed by applying 0.4 ml of pepsin (5mg/ml in 0.01 N HCl in 0.9% NaCl) (Sigma, St Louis, MO) at 37°C for 40 min. The slides were rinsed with distilled water for 3 min, and washed again with 2 x SSC for 5 min, and then air-dried. Dual color FISH was performed by using a mixture of Spectrum Orange labeled centromeric alpha satellite DNA probe (CEP12) and green labeled RP11-267D19 5-Fluorescein (12p11.21) DNA probes for chromosome 12p. Both of the probes were from Vysis (Downers Grove, IL) and were diluted with tDenHyb2 (Insitus, Albuquerque, NM) in a ratio of 1:50 and 1:20, respectively. Five microliters of diluted probes were added to the slide in the reduced light condition, slides were covered with a coverslip, and were sealed with rubber cement. Denaturation was achieved by incubating the slides at 75°C for 10 minutes in a humidified box, then the slides were hybridized at 37°C overnight. The coverslips were removed and the slides were washed extensively twice with 45°C prewarmed 0.1X SSC/1.5 mol/L urea, for 20 minutes for each, followed by a wash with 2X SSC for 20 minutes and 2X SSC/0.1% NP40 for 10

minutes at 45°C. The slides were further washed with room temperature 2X SSC for 5 minutes. The slides were air-dried and counterstained with 10 µL of 4,6-diamidino-2-phenylindole (DAPI AntifadeInsitus). The slides were covered and sealed with nail polish. The slides were examined using a Zeiss Axioplan 2 microscope with the following filters: SP-100 DAPI, FITC MF-101 for Spectrum Green (12p) and Gold 31003 for Spectrum Orange (CEP12) from Chroma (Brattleboro, VT). The images were acquired with a CCD camera and analyzed with MetaSystem Isis software (Belmont, MA). Five sequential focus stacks with 0.4-µm intervals were acquired and then integrated into a single image in order to reduce thickness-related artifacts. From each tumor section, 100 nuclei were scored for signals from CEP12 (red) and 12p (green) under the fluorescence microscope with 1,000X magnification, and the ratio between green and red signals was subsequently calculated. We analyzed the spatial distribution of the green and red signals to detect the specific patterns of signal aggregation consistent with i(12p), as previously reported.[37] The quantitative criteria to determine 12p overrepresentation have been described previously.[37] A classical seminoma specimen was used as a positive control for FISH analyses. Lymphocyte and stromal nuclei from the same tumor were used as normal controls for each tumor. In addition, we analyzed six cases of skin punch biopsies from patients without a history of germ cell tumors as negative controls.

Results

A total of 50 cases from 48 neonates, infants, and children (2 recurrent cases from 2 different patients) were identified. Thirty-four cases were from female (F) patients and 14 from male (M) (F: M ratio = 2.4: 1). Patient age ranged from 0 days–8 years (median, 2 weeks). Follow up information was available in 30 patients (range: 4–254 months; mean, 8.9 years), and

all were alive with no evidence of disease with the exception of one who was alive but disease status unknown.

Among the 48 pediatric patients, there were 44 teratomas and 4 tumors consisting of teratoma and yolk sac tumor (1 of which also had primitive neuroectodermal tumor). The teratomas included 37 mature teratomas and 11 immature teratomas (4 grade 1, 2 grade 2, and 5 grade 3). The 44 teratomas lacking a yolk sac tumor component were all negative for i(12p) (**Figure 1**). The 4 tumors with a yolk sac tumor component were all positive for i(12p) (**Figure 2**).

A total of four adult cases from 3 females and 1 male were identified (F: M ratio = 3: 1). Patient age ranged from 22–50 years (median, 35 years). Follow up information was available in 2 patients, and both were alive with no evidence of disease at 82 and 180 months, respectively. None of these patients had a documented history of teratoma in childhood, and all cases lacked nonteratomatous germ cell tumor components, immature elements, and i(12p).

Follow up information was, therefore, available for 32 patients. Two patients with teratoma had recurrence, but were alive with no evidence of disease after long-term follow up. One patient with teratoma and yolk sac tumor had recurrence 7 months after resection. The other patients were alive with no evidence of disease at last follow up.

A detailed clinicopathologic summary of all 54 cases including their i(12p) status is provided in Tables 1 and 2.

Discussion

Sacrococcygeal teratomas occurring in adults are rare, and there have been limited studies on the i(12p) status of sacrococcygeal teratomas, including those with malignant GCT

elements. We therefore investigated the i(12p) status of a large series of both prepubertal and postpubertal sacrococcygeal teratomas, some including yolk sac tumor and primitive neuroectodermal tumor, and correlated the findings with available long-term clinical follow up.

The majority (88%) of our sacrococcygeal teratomas occurred in infants (<1 year) with a female predominance (F: M = 2.4: 1, or 71% among the pediatric cases). These findings are similar to large American,[13] British,[15] and Japanese[17] series of pediatric sacrococcygeal GCT cases, in which 74% (of 126 patients), 80% (of 51 patients), and 75% (of 289 patients) were girls. There were 35 mature teratomas (lacking a yolk sac tumor component, 73%), 9 immature teratomas (lacking another component, 19%), and 4 teratomas with a yolk sac tumor component (8%). These findings are similar to those reported in the largest of these series, Yoshida, et al. (67% mature teratoma, 16% immature teratoma, and 17% with yolk sac tumor).[17]

All four pediatric cases with yolk sac tumor (including one which also had a primitive neuroectodermal tumor component) displayed i(12p). The other 44 pediatric cases (including 35 mature teratomas and 9 immature teratomas) lacked i(12p). Three of the 30 pediatric cases with follow up (10%) had recurrence, a percentage very similar to the 8.7% reported by Yoshida, et al.[17]

All four adult cases were negative for i(12p) and neither of the 2 patients with follow up had recurrence. As a whole, regardless of patient age, the presence of malignant GCT components, or i(12p) status, all patients except for one were alive without evidence of disease at time of last follow up (mean, 9.4 years).

The occurrence of teratomas in the sacrococcygeal region, representing approximately 40% of all teratomas, is not well understood, but may relate to the presence of stem cells. Evidence for this includes the expression of the stem cell markers NANOG, OCT4, SSEA-4, and

nestin in these tumors as well as the high risk of recurrence if the coccyx (thought to harbor associated stem cells) is not removed along with the tumor.[38] These tumors have been believed to arise from the embryonic tail bud,[38] but the presence of foregut-derived structures such as respiratory epithelium has been cited as arguing against this interpretation.[39] Sacrococcygeal teratomas most commonly occur in neonates and infants and studies consistently show a female predominance.[11, 13, 15] The reason for the female predominance has not been determined, but might result from later sex-specific differentiation of the caudal mesenchymal tissue including the vertebral column and the pelvic skeleton in the embryo.[38] Occasional examples of these tumors have been familial with known or suspected mutations, including patients with Currarino Syndrome, thought to involve the HLXB9 gene,[40] or with other genetic associations such as partial trisomy 3q with Cornelia de Lange Syndrome-like phenotype.[41] Most of the patients, however, do not have a known genetic abnormality.

The frequency of a malignant component within a sacrococcygeal teratoma is related to patient age, with 1.5% of tumors from newborns containing a malignant component compared to nearly 40% of tumors from children older than 1 year.[18] It has been suggested that yolk sac tumor may occur in sacrococcygeal teratoma as a form of tumor progression.[42] This seems to be true for the wide variety of somatic tumors that may arise in sacrococcygeal GCTs, including recently reported pediatric cases with sarcoma,[43] nephroblastic elements,[44] peripheral primitive neuroectodermal tumor,[40] malignant ependymoma component,[45] neuroblastoma,[46] and malignant steroidogenic tumor[47] and adult cases with neuroendocrine carcinoma,[48] adenocarcinoma,[49] and intestinal-type mucinous neoplasm which recurred as disseminated intraperitoneal disease with pseudomyxoma peritonei.[50]

Testicular teratomas may be behaviorally and pathogenetically separated into two broad groups: prepubertal teratomas with typically benign behavior and postpubertal teratomas that are typically components of malignant germ cell tumors. The prepubertal teratomas lack i(12p) abnormalities and are therefore thought to arise from germ cells that are cytogenetically normal, at least in terms of 12p amplification.[28, 51] The i(12p) abnormality or other 12p amplification is a nearly constant finding in adult testicular GCTs including pure teratoma,[51]; however occasional benign postpubertal testicular teratomas lacking 12p amplification exist.[52]

Ovarian teratomas without and with nonteratomatous elements similarly appear to represent two pathogenetically distinct categories.[36] Ovarian teratomas lack i(12p) abnormalities, even if immature teratoma is present, unless a nonteratomatous germ cell component is present.[28, 36] Ovarian teratoma with associated yolk sac tumor and/or embryonal carcinoma will frequently contain i(12p) in both the teratoma and nonteratomatous components.[36]

It seems reasonable to speculate that sacrococcygeal teratomas similarly may be divided into two pathogenetically distinct categories, but previously few data concerning i(12p) status were available. Using classical cytogenetic analysis, no chromosome 12 abnormalities were noted in 18 pediatric sacrococcygeal teratomas, including cases with immature teratoma but no nonteratomatous component (3 cases) and cases with nonteratomatous components (6 cases).[28] A series of 14 pediatric and 5 adult sacrococcygeal teratomas with i(12p) analysis by FISH was recently reported by Gurda, et al.[16] In that study, tumors with associated malignant germ cell tumor (yolk sac tumor) were excluded, but 3 of the pediatric cases had immature elements. While recurrence was observed in 3 pediatric cases and 4 adult cases, no mortality was documented with a median follow up of 6 years. Thirteen of these cases (9 pediatric and 4 adult) had FISH analysis performed and none of the cases had an i(12p) abnormality.

Our findings confirm the absence of i(12p) in both pediatric and adult sacrococcygeal teratoma cases, unless an associated malignant GCT component is present. Only 1 tumor with immature teratoma was analyzed for i(12p) in the previous study[16] and we have confirmed the absence of i(12p) in 9 additional cases of teratoma with an immature component but no malignant germ cell component.

The i(12p) abnormality was not observed in the 4 adult sacrococcygeal teratomas. The i(12p) abnormality was detected in 8% (4/44) of pediatric sacrococcygeal germ cell tumors, but only in those with a nonteratomatous component. This is similar to the findings in ovarian teratomas with and without a nonteratomatous component. These cytogenetic findings suggest that pediatric sacrococcygeal teratomas should be considered as two distinct groups, those with and those without other components, and that each group has a distinct molecular pathogenesis.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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Figure Legends

Figure 1: Mature teratoma with glial (1A), ciliated and enteric-type glandular epithelium (1B), and squamous epithelium (1C). FISH analysis (1D) shows two paired red and green signals per nucleus, indicating two intact copies of chromosome 12.

Figure 2: Tumor with mature teratoma (2A), immature teratoma (2C), and yolk sac tumor components (2E). FISH analysis demonstrates green-red-green signals indicating an i(12p) in the mature teratoma (2B), immature teratoma (2D), and yolk sac tumor components (2F).

Table 1. Clinicopathologic summary of sacrococcygeal teratomas in both pre- and post-pubertal patients

Patient	Sex	Age	Teratoma Grade	Other GCT	i(12p)	Duration of F/U (months)	F/U Status
Pediatric cases							
1	F	1d	0	None	Neg	N/A	N/A
2	F	1d	0	None	Neg	198	Alive, NED
3	F	2d	0	None	Neg	N/A	N/A
4	F	2d	0	None	Neg	163	Alive, NED
5	F	2d	0	None	Neg	N/A	N/A
6	F	2d	0	None	Neg	N/A	N/A
7	F	2d	1	None	Neg	N/A	N/A
8	F	2d	1	None	Neg	142	Alive, NED
9	F	2d	2	None	Neg	N/A	N/A
10	F	2d	3	**YST and PNET**	**Pos**	136	Alive, NED
11	F	4d	0	None	Neg	N/A	N/A
12	F	4d	0	None	Neg	N/A	N/A
13	F	4d	0	None	Neg	N/A	N/A
14	F	5d	0	None	Neg	77	Alive, NED
15	F	5d	3	None	Neg	21	Alive, NED
16	F	7d	0	None	Neg	140	Alive, NED
17	F	12d	2	None	Neg	59	Alive, NED
18	F	2w	0	None	Neg	136	Alive, NED
19	F	3w	0	**YST**	**Pos**	7	Recurred
20	F	4w	0	None	Neg	4	Alive, NED
21	F	6w	0	None	Neg	4	Alive, NED

22	F	6w	0	None	Neg	N/A	N/A
23	F	8w	0	None	Neg	48	Alive, NED
24	F	9w	0	None	Neg	90	Alive, NED
25	F	3m	0	None	Neg	243	Alive, NED
26	F	3m	3	None	Neg	199	Alive, NED
27	F	4m	0	None	Neg	N/A	N/A
28	F	5m	0	None	Neg	N/A	N/A
29	F	7m	1	**YST**	**Pos**	94	Alive, NED
30	F	1y	0	None	Neg	-	-
30	F	1y	0	None	Neg	188	Recurred 8 months later but now Alive, NED
31	F	1y	0	None	Neg	233	Alive, NED
32	F	1y	0	None	Neg	N/A	N/A
33	F	1y	0	**YST**	**Pos**	15	Alive
34	F	1y	0	None	Neg	52	Alive, NED
35	M	16w GA fetus	3	None	Neg	N/A	N/A
36	M	1d	0	None	Neg	N/A	N/A
37	M	2d	3	None	Neg	228	Alive, NED
38	M	4d	0	None	Neg	81	Alive, NED
39	M	4d	0	None	Neg	254	Alive, NED
40	M	11d	0	None	Neg	59	Alive, NED
41	M	2w	0	None	Neg	-	-
41	M	6m	0	None	Neg	147	Recurred after 6 months but now Alive, NED

42	M	3w	0	None	Neg	139	Alive, NED
43	M	4w	0	None	Neg	N/A	N/A
44	M	4w	1	None	Neg	35	Alive, NED
45	M	8m	0	None	Neg	N/A	N/A
46	M	1y	0	None	Neg	N/A	N/A
47	M	6y	0	None	Neg	77	Alive, NED
48	M	8y	0	None	Neg	95	Alive, NED
Adult cases							
49	F	35y	0	None	Neg	82	Alive, NED
50	F	35y	0	None	Neg	N/A	N/A
51	F	50y	0	None	Neg	180	Alive, NED
52	M	22y	0	None	Neg	N/A	N/A

Table 2. Summary of 52 patients (54 specimens) with sacrococcygeal teratoma.

	Pediatric patients (48)	Adult patients (4)
Female: Male Ratio	2.4 : 1	3 : 1
Age Range Median	 0-8 years 2 weeks	 22-50 years 35 years
Pathology and i(12p) status Teratoma (without YST), G0 Teratoma (without YST), G1 Teratoma (without YST), G2 Teratoma (without YST), G3 Teratoma and yolk sac tumor	 35 (0% i(12p)) 3 (0% i(12p)) 2 (0% i(12p)) 4 (0% i(12p)) 4 (100% i(12p))	 4 (0% i(12p))
Outcome No evidence of disease Alive (status unknown) Recurrent Not available	 28* 1 1 18	 2 2
Mean follow up (range)	8.9 years (4-254 months)	10.9 years (82-180 months)

*Including 2 with recurrence and second excision with NED at 147 and 188 months follow up.

YST: yolk sac tumor.

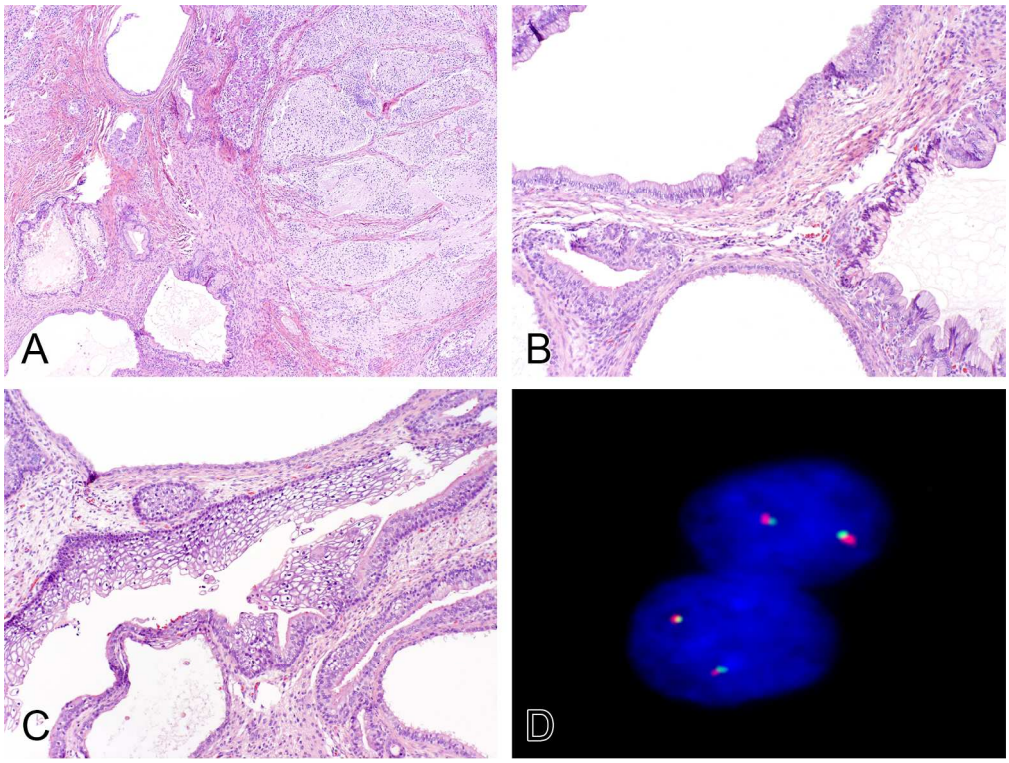

Figure 1
206x155mm (300 x 300 DPI)

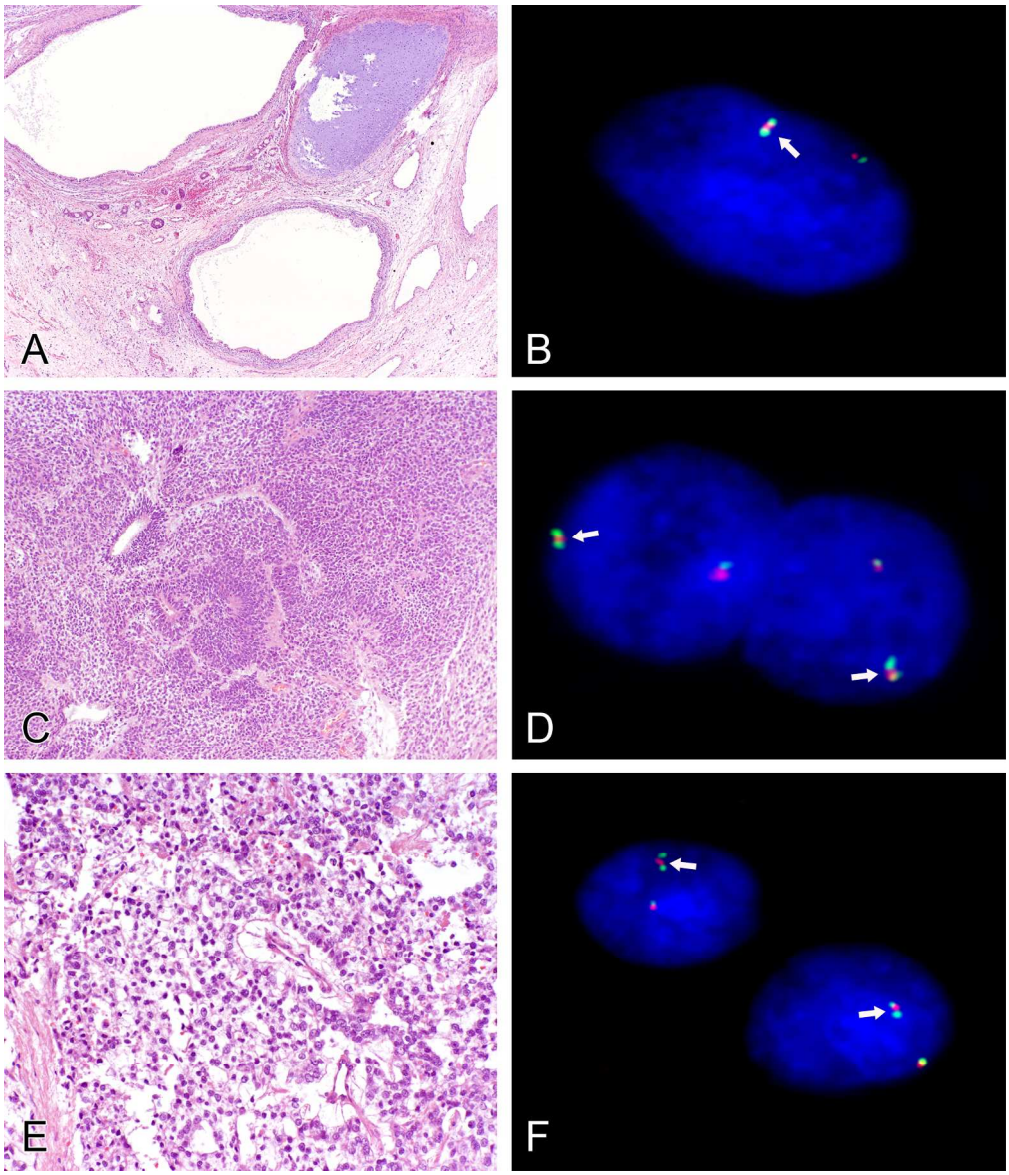

Figure 2
206x240mm (300 x 300 DPI)